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Proposed Registration Decision PRD2012-06

Trichoderma virens strain G-41

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Overview

Proposed Registration Decision for *Trichoderma virens* strain G-41

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of G-41 Technical and RootShield Plus Wettable Powder, containing the technical grade active ingredients *Trichoderma virens* strain G-41 and *Trichoderma hazianum* Rifai strain KRL-AG2, to suppress certain *Phytophthora* species that cause root rot on outdoor container-grown ornamentals.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

This Overview describes the key points of the evaluation, while the Science Evaluation section provides detailed technical information on the human health, environmental and value assessments of G-41 Technical and RootShield Plus Wettable Powder.

What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable[1] if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its proposed conditions of registration. The Act also requires that products have value[2] when used according to the label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (for example, children) as well as organisms in the environment (for example, those most sensitive to environmental contaminants). These methods and policies also consider the nature of the effects observed and the uncertainties when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the PMRA's website at healthcanada.gc.ca/pmra.

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1 "Acceptable risks" as defined by subsection 2(2) of the *Pest Control Products Act*.

2 "Value" as defined by subsection 2(1) of the *Pest Control Products Act*: "the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's (*a*) efficacy; (*b*) effect on host organisms in connection with which it is intended to be used; and (*c*) health, safety and environmental benefits and social and economic impact."

Before making a final registration decision on *Trichoderma virens* strain G-41, the PMRA will consider all comments received from the public in response to this consultation document.[3] The PMRA will then publish a Registration Decision[4] on *Trichoderma virens* strain G-41, which will include the decision, the reasons for it, a summary of comments received on the proposed final registration decision and the PMRA's response to these comments.

For more details on the information presented in this Overview, please refer to the Science Evaluation section of this consultation document.

What Is *Trichoderma virens* strain G-41?

Trichoderma virens strain G-41 a naturally occurring soil fungus that was isolated from soils in North America. It occupies a special niche on the roots of plants but can be found on decaying wood and vegetable matter. *Trichoderma virens* strain G-41 is used as a microbial pest control agent (MPCA) in G-41 Technical, and the associated end-use product, RootShield Plus Wettable Powder. RootShield Plus Wettable Powder is a commercial fungicide which contains live spores of the MPCA, and is proposed for use to protect the roots of outdoor container-grown ornamentals/nursery plants against root rot fungal pathogens (for example, *Phytophthora parasitica* and *Phytophthora cinnamomi*).

When applied to soil, the MPCA actively grow on the developing plant roots. There appears to be several methods of antagonism towards pathogens. Systemic resistance appears to be induced in response to the production of various fungal metabolites, particularly gliotoxin. Mycoparasitism of fungal pathogens and competition for resources also play a role in antibiosis of the fungal pathogens.

In addition to *T. virens* strain G-41, the end-use product also contains the registered MPCA *Trichoderma harzianum* strain KRL-AG2 (Registration number 27114; 5.0%; 1.0×10^7 CFU/g dry wt).

Health Considerations

Can Approved Uses of *Trichoderma virens* strain G-41 Affect Human Health?

***Trichoderma virens* strain G-41 is unlikely to affect your health when RootShield Plus Wettable Powder is used according to the label directions.**

Exposure to *Trichoderma virens* strain G-41 may occur during handling of RootShield Plus Wettable Powder.

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3 "Consultation statement" as required by subsection 28(2) of the *Pest Control Products Act*.

4 "Decision statement" as required by subsection 28(5) of the *Pest Control Products Act*.

When assessing health risks, several key factors are considered:

- the microorganism's biological properties (for example, production of toxic byproducts);
- reports of any adverse incidents;
- its potential to cause disease or toxicity as determined in toxicological studies; and
- the levels to which people may be exposed relative to exposures already encountered in nature to other isolates of this microorganism.

Toxicological studies in laboratory animals describe potential health effects from large doses in order to identify any potential pathogenicity, infectivity and toxicity concerns.

When *Trichoderma virens* strain G-41 was tested on laboratory animals, there were no signs that it caused any toxicity or disease.

The end-use product, RootShield Plus Wettable Powder was not a dermal irritant, but was mildly irritating to the eyes. Workers primarily exposed to the product will be required to wear standard personal protective equipment including eye goggles to reduce the potential for dermal and ocular exposure.

Like all microbial pest control agents, *Trichoderma virens* strain G-41 contains substances that can cause allergic reactions in people who are repeatedly exposed to it at high concentrations. G-41 Technical and RootShield Plus Wettable Powder also contain the allergens, wheat and sulphites. However, allergic reactions can be avoided if commercial applicators follow label recommendations to minimize or limit exposure to RootShield Plus Wettable Powder.

Residues in Water and Food

Dietary risks from food and water are not of concern.

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally established as a maximum residue limit (MRL) under the *Pest Control Products Act* for the purposes of the adulteration provision of the *Food and Drugs Act* (FDA). Health Canada sets science-based MRLs to ensure the food Canadians eat is safe.

As there are no direct applications to food, there is no concern for risks posed by dietary exposure of the general population, including infants and children, or animals. Consequently, the establishment of an MRL is not required for *Trichoderma virens* strain G-41. As well, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Consequently, dietary risks are minimal to non-existent.

Occupational Risks From Handling RootShield Plus Wettable Powder

Occupational risks are not of concern when RootShield Plus Wettable Powder is used according to label directions, which include protective measures.

Workers using RootShield Plus Wettable Powder can come into direct contact with *Trichoderma virens* strain G-41 on the skin, by inhalation, or in the eyes. For this reason, the label will specify that users exposed to RootShield Plus Wettable Powder must wear waterproof gloves, long-sleeved shirts, long pants, and shoes plus socks, eye goggles, and a dust/mist filtering NIOSH approved respirator/mask (with any N-95, P-95, R-95 or HE filter) until mists have settled and during clean-up and repair activities. After application of RootShield Plus Wettable Powder, workers will be cautioned to avoid skin contact with treated soil/potting mix.

For bystanders, exposure is expected to be much less than that of workers involved in loading and application activities and is considered negligible. Therefore, health risks to bystanders are not of concern.

Environmental Considerations

What Happens When RootShield Plus Wettable Powder Is Introduced Into the Environment?

Environmental risks are not of concern.

Following application, *Trichoderma virens* strain G-41 is likely to survive in the environment under favourable environmental conditions (in other words, temperature, humidity) but over time populations of *T. virens* strain G-41 are expected to return to natural background levels.

There are no published reports of disease associated with *Trichoderma virens* strain G-41 in birds, wild mammals, fish, insects or plants, except for the intended pest.

Also, minimal exposure to non-target organisms is anticipated from the proposed use of RootShield Plus Wettable Powder as a soil drench to control root rot in outdoor container-grown ornamentals.

Value Considerations

What Is the Value of RootShield Plus Wettable Powder?

RootShield Plus Wettable Powder contains *Trichoderma virens* strain G-41 and *T. harzianum* Rifai strain KRL-AG2, which is currently registered as RootShield to suppress root pathogens on outdoor container-grown ornamentals.

T. virens G-41 has been combined with *T. harzianum* KRL-AG2 to suppress *Phytophthora* root pathogens on ornamental crops.

Measures to Minimize Risk

Labels of registered pesticide products include specific instructions for use. Directions include risk-reduction measures to protect human and environmental health. These directions must be followed by law.

The key risk-reduction measures being proposed on the label of RootShield Plus Wettable Powder to address the potential risks identified in this assessment are as follows.

Key Risk-Reduction Measures

Human Health

Although the potential for toxicity is low in individuals exposed to *Trichoderma virens* strain G-41, sensitization may occur upon repeated exposure to high concentrations of the product. A label statement warning users that the product is a potential sensitizer is required. G-41 Technical and RootShield Plus Wettable Powder contain the allergen, wheat and RootShield Plus Wettable Powder also contains the allergen, sulphites. Consequently, label statements warning users as to the presence of these allergens are required on the labels for G-41 Technical and RootShield Plus Wettable Powder. To minimize exposure to mists generated while handling or applying RootShield Plus Wettable Powder, applicators, mixer/loaders, and handlers will be required to wear personal protective equipment, including a long-sleeved shirt, long pants, shoes plus socks, waterproof gloves, eye goggles, and a dust/mist filtering respirator (MSHA/NIOSH approval number prefix TC-21C) or a NIOSH approved respirator with any N-95, R-95, P-95 or HE filter for biological products until mists have settled and during all clean-up/repair activities. After application, workers will be cautioned to avoid skin contact with treated soil/potting mix. A label statement will also warn users that RootShield Plus Wettable Powder is a mild eye irritant.

Environment

As a general precaution, the label prohibits the direct application of the product to aquatic habitats (such as lakes, streams and ponds). The label also directs users to avoid contaminating surface water by disposal of equipment wash waters.

Next Steps

Before making a final registration decision on *Trichoderma virens* strain G-41, the PMRA will consider all comments received from the public in response to this consultation document. The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications (contact information on the cover page of this document). The PMRA will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed final decision and the Agency's response to these comments.

Other Information

When the PMRA makes its registration decision, it will publish a Registration Decision on *Trichoderma virens* strain G-41 (based on the Science Evaluation section of this consultation document). In addition, the test data referenced in this consultation document will be available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa).

Science Evaluation

Trichoderma virens strain G-41

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active microorganism	*Trichoderma virens*
Function	To provide protection to the roots of outdoor container-grown nursery plants against root rot fungal pathogens *(Phytophthora cinnamomi* and *Phytopthora parasitica)*
Binomial name	*Trichoderma virens* strain G-41
Taxonomic designation	
Kingdom	Fungi
Phylum	Ducteromycontina
Order	Hyphomycetes
Genus	*Trichoderma*
Species	*virens*
Strain	G-41
Patent Status information	US Patent and Trademark Office (Patent number US 2010/0028303). The registrant has also filed an international patent application through the PCT (Patent Cooperation Treaty) and plans to file a national patent application in Canada.
Minimum purity of active	*Trichoderma virens* G-41 $\geq 1.0 \times 10^8$ CFU per gram dry weight
Identity of relevant impurities of toxicological, environmental and/or significance.	The TGAI does not contain any impurities or microcontaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. The product must meet microbiological contaminants release standards. *Trichoderma virens* strain G-41 is a known producer of gliotoxin, a secondary metabolite of toxicological significance. Gliotoxin has been confirmed to be present in the technical product and end-use product at relatively high levels; however, these levels are unlikely to affect human health when using the product according to its label directions.

1.2 Physical and Chemical Properties of the Active Ingredients and End-Use Product

Technical Grade Active Ingredient –G-41 Technical

Physical state	solid
colour	Gray green
odour	Slightly musty
pH	5.6 (1% solution)
Guarantee	$\geq 1.0 \times 10^8$ CFU/g dry weight

End-Use – RootShield Plus Wettable Powder

Physical state	solid
Colour	-
Odor	-
Density	-
pH	7.09
Guarantee	$\geq 5.0 \times 10^6$ CFU/g dry weight

1.3 Directions for Use

RootShield Plus Wettable Powder is applied directly to potting mix or as a soil drench.

Potting Mix: Suspend RootShield Plus Wettable Powder in sufficient water to achieve uniform application (for example 30 – 40 g/100 L). Apply solution to (loose) greenhouse potting mix, soil or planting beds at a rate of 30 – 40 g Rootshield Plus Wettable Powder per cubic metre.

Greenhouse and Nursery Drench: Suspend RootShield Plus Wettable Powder in sufficient water (eg. 30 – 40 g/100 L) to achieve uniform application and apply as follows:

Type of growing container or bed	Amount of RootShield Plus Wettable Powder per square meter
Shallow flats and pots up to 10 cm deep	2.5 L
Planting beds or pots deeper than 10 cm	10 L (or use per pot volumes shown in chart below)

RootShield Plus Wettable Powder Drench Volume Per Pot	
Pot Size (diameter)	**Amount of RootShield Plus Wettable Powder per pot**
13 cm	200 mL
15 cm	236 mL
20 cm	325 mL
25 cm	414 mL
30 cm	0.5 L
Above 30 cm use 1.0 L per 0.1 m^2	
Pot Size (volume)	**Amount of RootShield Plus Wettable Powder per pot**
3.7 L	355 mL
7.5 L	0.5 L
11.4 L	0.9 L
15 L	1.2 L
19 L	1.6 L
Above 19 L use 1.0 L per 0.1 m^2	

RootShield Plus Wettable Powder can be applied through low pressure watering nozzles such as fan nozzles or other watering systems. Agitate to maintain suspension.

1.4 Mode of Action

The mode of action of *Trichoderma virens* strain G-41 includes competition for food and space, mycoparasitism via the production of enzymes and antibiotics, and by the induction of plant defence responses. Both *Trichoderma* species secrete antibiotics and cell wall degrading enzymes including β-1,3-glucanases, chitinases and proteinases. They are also known to produce diterpenes, butenolides, pyrones, pyridines, peptaibols, and furanones which may contribute to the activity.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganism

Appropriate methodologies for detection, isolation and enumeration of the active ingredient, *Trichoderma virens* strain G-41strain were submitted. The MPCA has been fully characterized with respect to the origin of the strain, natural occurrence and biological properties. The identification of *T. virens* to the species level is achieved using standard mycological techniques for this genus. Additional tests to distinguish strain G-41 from other *Trichoderma* species and strains of *T. virens* include colony and morphology characteristics on differential growth medium developed for strain recognition. The taxonomic position of G-41 was confirmed by microscopic examination of asexual reproductive structures (phialides and conidiospores) according to Rifai's species description.

Trichoderma virens colonies after 3–4 days of incubation at 27°C (under constant fluorescent lighting) on potato dextrose agar (PDA) amended with 1mL/L Igepal Co 630 and 100 ppm Tartaric acid have a poorly defined colony perimeter, and are pale white with dark green condia, producing no pigment below the media. This lack of pigment production is exploited to differentiate between strain G-41 and colonies of *T. harzianum* strain KRL-AG2 which appear smaller relative to *T. virens* strain G-41 colonies, and circular in shape with a clearly defined perimeter, and diffuses a yellow pigment in the agar directly below the colony. Conidia of *T. harzianum* strain KRL-AG2 are pale green in colour.

The presence of gliotoxin, a secondary metabolite of *T. virens* strain G-41 has been confirmed at considerably high levels in the technical and end-use product. The PMRA has concluded that the HPLC analysis method employed in the detection and quantification of gliotoxin needs additional refinements to further improve its accuracy; however, based on the proposed ornamental use, the presence of gliotoxin is unlikely to affect human health when using the product according to its label directions.

2.2 Methods for Establishment of Purity of Seed Stock

Working cultures of *T. virens* strain G-41 are prepared annually and stored on silica grains at −20°C to enable later manufacture of inoculums. Quality assurance is performed to determine the potency of the inoculum and to confirm the absence of microbial contaminants. Potential bacterial contaminants are monitored by plating dilutions of samples onto tryptic soy agar (TSA) whereas fungal contaminants are monitored using potato dextrose agar (PDA). Any bacterial or fungal contamination will result in rejection of working cultures.

2.3 Methods to Define the Content of the Microorganism in the Manufactured Material Used for the Production of Formulated Products

The colony forming units (CFU) Test is a standardized method to determine viability of the active ingredient and performed to estimate the number of viable propagules of strain G-41 per unit mass of sample (CFU/g dry weight). The guarantee of the TGAI is based on the number of colony forming units per gram of product. Dilutions of the product are spread onto the surface of tryptic soy agar plates and the resulting colonies are counted in order to calculate the viable cell count.

2.4 Methods to Determine and Quantify Residues (Viable or Non-viable) of the Active Microorganism and Relevant Metabolites

As the end-use product has not been approved for food uses, no methods to determine and quantify the MPCA and relevant metabolites are required.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

During manufacturing, several approaches will be used to monitor microbial contamination in the technical product and the associated end-use product. These approaches will include frequent purity checks on agar media, sterilization of all equipment and media, and sanitization of recovery equipment.

The absence of human pathogens and below-threshold levels of contaminants was demonstrated in representative batches using pathogen-specific growth media using acceptable methods. Release standards for microbial contaminants in the production batches comply with those permitted by the PMRA and are adequate to ensure that the end-use product does not contain unacceptable levels of human and animal disease-causing microorganisms.

2.6 Methods to Determine Storage Stability, Shelf-life of the Microorganism

The viability of *T.virens* strain G-41 in RootShield Plus Wettable Powder was evaluated over a 6 month period at 4°C and at 21°C. The submitted storage stability data support a storage period for RootShield Plus Wettable Powder of up to 6 months at 23 °C.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

The PMRA conducted a detailed review of the toxicological database for G-41 Technical. The database for G-41 Technical, containing the MPCA *Trichoderma virens* strain G-41, is complete, consisting of laboratory animal (in vivo) toxicity studies (acute oral toxicity/pathogenicity and acute intraperitoneal infectivity) currently required for health hazard assessment purposes. A waiver was considered acceptable to address the acute pulmonary toxicity/pathogenicity of the MPCA in lieu of testing. Dermal toxicity, dermal irritation and eye irritation studies were also submitted to address these requirements for the end-use product, RootShield Plus Wettable Powder, and a waiver was considered acceptable to address its potential for hypersensitivity.

In addition to these required studies, several non-compulsory studies were also conducted. These include an oral toxicity study, an inhalation toxicity study, and an eye irritation study with RootShield Plus Wettable Powder. Furthermore, an oral toxicity/pathogenicity study, a pulmonary toxicity/pathogenicity study, and an intravenous infectivity study with *Gliocladium virens* strain GL-21 (*Trichoderma virens* strain GL-21) were also submitted. *Trichoderma virens* strain GL-21 is a strain closely related to the MPCA which is currently registered in the United States in another end-use product. A study to compare the MPCA (*Trichoderma virens* strain G-41) and *Trichoderma virens* strain GL-21 demonstrated that the two strains are functionally very similar to one another and overall, based on the similarity of the two strains, it is reasonable to expect that toxicological data on strain GL-21 would be representative of the toxicity of G-41 (PMRA 1988115). Therefore, the studies were considered acceptable as supplemental information on the toxicity of *Trichoderma virens* strain G-41.

In the acute oral, inhalation, and dermal toxicity studies, and the dermal and eye irritation studies conducted with RootShield Plus Wettable Powder and summarized below, the RootShield Plus Wettable Powder test substance contained 0.6% *Trichoderma virens* strain G-41 and 1.15% *T. harzianum* strain KRL-AG2 rather than the 9.5% *T. virens* and 5.0% *T. harzianum* in the end-use product formulation proposed for registration. Also, potency (in other words, CFU/g dry wt) of the test substance was not provided in the study reports. These studies were considered to be acceptable because of the completeness of the overall toxicity database and the expectation that the label warning statements and precautionary measures will mitigate any potential occupational risks from the end-use product.

Overall, the studies were carried out in accordance with currently accepted international testing protocols and good laboratory practices. The scientific quality of the data is high and the database is considered sufficient to characterize the toxicity and infectivity of this pest control agent and product.

In an oral toxicity and infectivity study (PMRA 2003619), fasted, young adult Sprague-Dawley rats (12/sex) were given a single oral dose of G-41 Technical (1.5×10^9 CFU/mL) in phosphate buffered saline at $>10^8$ CFU/animal in a dosing volume of 0.1 mL. An untreated control group (5/sex), a shelf control group (2/sex), and an inactivated test substance group (5/sex) were conducted concurrently. The animals were observed for a period of up to 21 days with interim scheduled sacrifices on Days 3, 7 and 14. There were no mortalities throughout the study, no signs of toxicological effects or abnormal findings upon necropsy, and body weight was largely unaffected. Enumeration of the test substance confirmed its presence in the fecal samples on Day 1, and counts were below clearance threshold in the brain, lungs, liver, spleen, kidneys, and lymph nodes by Day 3, and by Day 7 for the cecum contents. However, as the sensitivity of detection demonstrated a low recovery of the MPCA from tissues and fluids (<10%), pathogenicity cannot be considered unequivocally assessed. Based on the results from this study, *Trichoderma virens* strain G-41 is of low toxicity at 10^8 CFU/animal via the oral route.

In an oral toxicity and infectivity study (PMRA 1979843), fasted, young adult Sprague-Dawley rats (13/sex) were given a single oral dose of *Trichoderma virens* strain GL-21 (a toxicologically similar strain to the MPCA) in carbylmethyl cellulose at $>10^8$ CFU/animal in a maximum dosing volume of 5.0 mL. An untreated control group (13/sex) was conducted concurrently. The animals were observed for a period of up to 14 days with interim scheduled sacrifices on Days 1, and 7. There were no mortalities, no effects on body weight and no abnormal findings upon necropsy. General signs of toxicity (lethargy, rapid breathing) were observed 1-hour post treatment, but all signs had resolved by Day 1. The test substance was detected in the fecal samples on Day 1, and in low levels in the lungs (one animal) on Day 7 but had cleared from all organs/tissues by Day 14. Based on the results from this study, *Trichoderma virens* strain GL-21 is of low toxicity and is not infective at 10^8 CFU/animal via the oral route.

In an oral toxicity study (PMRA 1807270), three fasted, young adult female Sprague-Dawley rats were given a single oral dose of RootShield Plus Wettable Powder in deionized water at >5000 mg/kg bw. The animals were observed for a period of up to 14 days. There were no mortalities throughout the study, no signs of toxicological effects, and body weight was largely unaffected. Based on the results from this study, RootShield Plus Wettable Powder is of low toxicity at 5000 mg/kg.

In a pulmonary toxicity and infectivity study (PMRA 1979846), fasted, young adult Sprague-Dawley rats (15/sex) were exposed by the intratracheal route to *Trichoderma virens* strain GL-21 (a toxicologically similar strain to the MPCA) at 10^8 CFU/animal in a dosing volume of 0.04 mL of saline. An untreated control group (15/sex) was conducted concurrently. The animals were observed for a period of up to 21 days with interim sacrifices on Days 1, 7, and 14. There were no mortalities throughout the study. General signs of toxicity (poor weight gain; lethargy; rapid breathing; rough hair coat) were observed in all animals after dosing, but all signs were resolved by Day 4 or sooner. The test substance was detected in the kidneys, brain, spleen, liver, lungs, blood and lymph nodes on Day 1. By Day 7, the test substance had cleared from all tissues/organs, except for the spleen in which low counts were detected until Day 14, and the lungs which still confirmed the presence of the test substance at study termination (Day 21). Although complete clearance of the test substance was not observed, a pattern of clearance was adequately established. Necropsy revealed grey spots in the lungs and enlarged, pale peritracheal lymph nodes of the majority of the treated animals at each interim sacrifice. Since signs of toxicity were resolved by Day 4, and the test substance was still present in the lungs until Day 21, the toxicity observed and accompanying necropsy findings appear to be related to administration of a bolus material via the intratracheal route, and is not likely to be an indication of toxicity of the test substance itself. Overall, while the observed toxicity effects and necropsy findings can be attributed to administration of a bolus material via the intratracheal route, the test animals rapidly recovered from these effects and a pattern of clearance of the test substance was established. Therefore, based on the results of this study, *Trichoderma virens* strain GL-21 is of low toxicity, and is not considered infective at 10^8 CFU/animal via the intratracheal route.

In an acute inhalation toxicity study (PMRA 1807271), young adult Sprague Dawley rats (5/sex) were exposed by the inhalation route to RootShield Plus Wettable Powder for 4 hours by nose-only at a concentration of 5.14 mg/L. Animals were observed for 14 days. There were no mortalities during the study, no effects on body weight, and no gross abnormalities noted upon necropsy. Clinical signs of general toxicity (decreased activity; piloerection) were resolved by Day 2. Based on these results, RootShield Plus Wettable Powder is of low toxicity at 5.14 mg/L via the inhalation route.

In an acute intraperitoneal infectivity study (PMRA 1986053), young adult Wistar rats (3/sex) were injected with G-41 Technical (9.6×10^6 CFU/g) in distilled water at a dose of 10^7 CFU/animal in a constant dosing volume of 1 mL. A vehicle control group (3/sex) was conducted concurrently. Animals were observed for 21 days. There were no mortalities, no treatment related clinical signs, no abnormal necropsy findings, and no effect on body weight. Based on these results, G-41 Technical is of low toxicity and is not pathogenic at 10^7 CFU/animal via the intraperitoneal route.

In an acute intravenous infectivity study (PMRA 1979848), young adult Sprague Dawley rats (15/sex) were injected with mycelia of *Trichoderma virens* strain GL-21 (potency not measured; a toxicologically similar strain to the MPCA) at a dose of 0.1 g/animal in a constant dosing volume of 0.5 mL of saline. An untreated control group (15/sex) was conducted concurrently. Animals were observed for up to 21 days with interim sacrifices on Days 1, 7, and 14. Eight of the thirty animals died by Day 2. Clinical signs observed in most of the surviving animals lasted until Day 8 and included lethargy, rough hair coat, hunched posture, and rapid breathing. These signs gradually diminished and by Day 9 all surviving animals appeared normal until the end of the study. Body weight was largely unaffected. Necropsy of animals that died on Day 1–2 revealed discoloured lungs (slightly red to dark red), stiffened heart (two animals), and a fluid-filled thoracic cavity (one animal). On Day 0 (day of dosing), the test organism was detected in high numbers in the lungs, and in low numbers in the spleen, liver, kidney, brain, and lymph node. The test organisms had cleared in all organs, except for the spleen, by Day 14 or sooner. By Day 21, the test substance had also cleared from the spleen. The test organism was not detected in the blood at any time point. Based on the necropsy and microbial enumeration findings, the observed mortalities were considered to be a result of mechanical damage (in other words, clogging of capillaries in lungs) from injection of the cell mass rather than from pathogenicity of the microorganism. Overall mycelia of *Trichoderma virens* strain GL-21 was considered toxic, but not infective, in rats at 0.1g/animal via the intravenous route.

A request to waive acute pulmonary toxicity and pathogenicity testing with the TGAI (PMRA 1986042, PMRA 1986046) was accepted based on a lack of toxicity of RootShield Plus Wettable Powder via the inhalation route (PMRA 1807271), and a lack of toxicity of *Trichoderma virens* strain G-41 via the intraperitoneal route (PMRA 1986053). Furthermore, *Trichoderma virens* strain GL-21, a toxicologically similar strain to *Trichoderma virens* strain G-41, demonstrated a lack of toxicity and pathogenicity via the intratracheal route (PMRA 1979846). Overall, the potential for acute pulmonary toxicity and pathogenicity of *Trichoderma virens* strain G-41 is adequately addressed by the available information available, and therefore no further testing was required.

In a dermal toxicity study (PMRA1807268), groups of young adult Sprague Dawley rats (5/sex) were dermally exposed to a suspension of RootShield Plus Wettable Powder (1 g/mL of deionized water) at 5050 mg/kg bw (5.0 mL dosing volume) for 24 hours to an area of approximately 10% of the body surface. Following exposure, the animals were observed for 14 days. There were no mortalities, no treatment related clinical signs, no necropsy findings, and no major changes in body weight. There were no signs of dermal irritation at any time throughout the study. Based on these results, RootShield Plus Wettable Powder is of low toxicity at 5050 mg/kg bw via the dermal route.

In a primary dermal irritation study (PMRA 1807269), New Zealand White rabbits (2 male; 1 female) were dermally exposed to 500 mg of RootShield Plus Wettable Powder in 0.5 mL of deionized water. The treatment was covered with a semi-permeable dressing for 4 hours. Observations for dermal irritation were made at 1, 24, 48 and 72 hours after removal of the dressing. There were no signs of dermal irritation at any point throughout the study. Based on these results, RootShield Plus Wettable Powder is non-irritating to the skin.

In a primary eye irritation study (PMRA 1807272), 57.3 mg of RootShield Plus Wettable Powder in 0.1 mL of deionized water was instilled into the conjunctival sac of the right eye of three young adult New Zealand White rabbits (1 male; 2 female). Eyes of treated animals were examined for 7 days and scored for ocular irritation. At 24-hours after treatment corneal opacity with redness, chemosis and discharge of the conjunctivae were observed in all animals. Redness and chemosis persisted through to the 72-hour (Day 3) timepoint but all positive effects had cleared by Day 4. Based on the Mean Average Score (MAS) for irritation of 13.78, and considering that some ocular irritation was still apparent at 72-hours, RootShield Plus Wettable Powder is considered a mild eye irritant. The standard precautionary statement "CAUTION-Eye Irritant" must be included on the end-use product label.

A request to waive a skin sensitization study (PMRA 1986061) was accepted based on the natural occurrence of the MPCA as a ubiquitous soil fungus, and on the nature of the inert ingredients in RootShield Plus Wettable Powder, as well as the lack of incidents of hypersensitivity or other adverse effects throughout the research, development, or testing of G-41 Technical. Nevertheless, because all microorganisms contain substances that could elicit positive hypersensitivity reactions in humans, *Trichoderma virens* strain G-41 is considered to be a potential sensitizing agent. Consequently the signals words "POTENTIAL SENSITIZER" are required on the principal display panels of G-41 Technical and RootShield Plus Wettable Powder labels. Furthermore, the label for RootShield Plus Wettable Powder must indicate that the product contains the allergens wheat and sulphites.

A survey of published literature conducted as part of the Product Characterization and Analysis for the MPCA indicated that *Trichoderma* species in general are not considered animal pathogens, and infections in humans from any *Trichoderma* species are rare. There were no reports in published literature of any infections in humans or other animals related to *Trichoderma virens*.

Higher tier subchronic and chronic toxicity studies were not required because of the low acute toxicity of the MPCA, and no indications of infectivity or pathogenicity in the test animals treated in the Tier I acute oral and pulmonary toxicity/infectivity tests.

Within the available scientific literature, there are no reports that suggest *Trichoderma virens* has the potential to cause adverse effects on the endocrine system of animals. The submitted toxicity/infectivity studies in the rodent indicate that, following oral and pulmonary routes of exposure, the immune system is still intact and able to process and clear the MPCA. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated for *Trichoderma virens* strain G-41.

Trichoderma virens strain G-41 is a known producer of the secondary metabolite gliotoxin. Although the high pressure liquid chromatography (HPLC) quantification method resulted in significant inter-sample variability from within the same lots, gliotoxin is expected to be present at considerably high levels in both the TGAI and the EP. Furthermore, the mode of action of the EP is partially attributed to induction of systemic resistance in response to gliotoxin. Given that the MPCA is expected to actively grow on the developing plant roots following application, it is expected that additional gliotoxin will be produced after application of RootShield Plus Wettable Powder to soil.

Although limited, the information in the published literature indicates that gliotoxin may pose a health concern with the liver appearing to be the target organ. A single oral administration of gliotoxin to hamsters at a dose of 15, 25, or 35 mg/kg bw resulted in mortality in 4/5, 5/8, and 7/8 animals, respectively (PMRA 2117514). The LD_{50}s in mice exposed to gliotoxin via the intraperitoneal (IP) and intravenous routes were found to be 25 mg/kg bw and 7.8 mg/kg bw, respectively. In rats, the LD_{50} for IP exposure was 50–65 mg/kg bw (PMRA 2120864). No information was available on the potential toxicity of gliotoxin via the dermal route and no chronic studies were available in the published literature. An endpoint of concern could not be established; however, occupational exposure to gliotoxin is not expected to pose a health concern based on the requirement for personal protective equipment, particularly the dust/mist filtering respirator for biological products and the instructions to avoid skin contact with treated soil or potting mix. In addition, the health risk to the general population is expected to be minimal since the EP will only be applied to container-grown outdoor ornamentals and is not permitted to be applied to residential or recreational areas or to food or feed crops.

3.2 Occupational/Bystander Exposure and Risk Assessment

3.2.1 Occupational

RootShield Plus Wettable Powder is a powder formulation which is applied as an aqueous suspension to loose potting mix or as a soil drench using standard watering or irrigation equipment. The treated growing media may also be handled post-application. When used according to the proposed label instructions, the potential routes of worker exposure to RootShield Plus Wettable Powder, containing the active ingredient *Trichoderma virens* strain G-41, are dermal, pulmonary and to some extent ocular. However, the PMRA does not expect that the occupational exposure from the proposed uses will be of concern on the basis of the low toxicity/pathogenicity profile for *T. virens* strain G-41, and on the assumption that the precautionary labelling instructions aimed at minimizing worker exposure are adhered to by users.

Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, or if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. This MPCA has not been identified as a wound pathogen and there is no indication that it could penetrate intact skin of healthy individuals. Based on the toxicological profile for *Trichoderma virens* strain G-41, no dermal toxicity or irritation is expected from exposure to RootShield Plus Wettable Powder.

As the end-use product is a wettable powder, inhalation exposure to workers from dust is primarily likely to arise during mixing activities, particularly when the product is mixed with water prior to application to soil. Inhalation exposure once the product is suspended in water is expected to be minimal as the product is applied through standard watering or irrigation equipment. Based on the toxicological profile for *Trichoderma virens* strain G-41, exposure to a large single quantity of the MPCA via the pulmonary route is not of concern.

Based on the results of the eye irritancy study, RootShield Plus Wettable Powder is expected to cause mild eye irritation on exposure, with the effects being reversible. As the end-use formulation being proposed for registration is a wettable powder, the potential for ocular exposure is low with the greatest potential for ocular exposure occurring during loading activities. The precautionary statement on the technical and end-use product labels will also warn users of the potential for eye irritation (Caution: Eye Irritant).

Although the potential for toxicity is low based on toxicological studies of the MPCA and toxicological characteristics of the ingredients present in the end-use formulation, sensitization may occur upon repeated exposure to high concentrations of the product, since the PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions. As all MPCAs are considered potential sensitizers, a label statement warning users that the product is a potential sensitizer is required. To minimize exposure to dust and mists while handling or applying the product, applicators, mixer/loaders, and handlers will be required to wear personal protective equipment, including a long-sleeved shirt, long pants, shoes plus socks, waterproof gloves, and a dust/mist filtering respirator (MSHA/NIOSH approval number prefix TC-21C) or a NIOSH approved respirator with any N-95, R-95, P-95 or HE filter for biological products until spray mists have settled and during all clean-up/repair activities.

Gliotoxin, a secondary metabolite of the MPCA, is known to be in the TGAI and end-use product. Occupational exposure is expected to be greatest during mixing activities, such as when the product is suspended in water and applied to potting mix or as a soil drench. Occupational exposure to gliotoxin is not expected to pose a health concern based on the results of human health and safety studies on the TGAI and EP as well as the requirement for personal protective equipment, including a long-sleeved shirt, long pants, shoes plus socks, waterproof gloves, eye goggles, and a dust/mist filtering respirator (MSHA/NIOSH approval number prefix TC-21C) or a NIOSH approved respirator with any N-95, R-95, P-95 or HE filter for biological products. To further reduce exposure to gliotoxin, language appears on the RootShield Plus Wettable Powder label advising workers to avoid contact of the treated soil/potting mix with skin.

3.2.2 Bystander

Inhalation or dermal exposure to the general public is expected to be low based on the proposed commercial application of RootShield Plus Wettable Powder to potting mix and soil in outdoor container-grown ornamentals. Overall the PMRA does not expect that bystander exposure will pose an undue risk on the basis of the low toxicity/pathogenicity profile for G-41 Technical and RootShield Plus Wettable Powder.

Bystander exposure to gliotoxin is also not expected to pose an undue risk based on the use pattern.

The label does not allow applications to turf, residential or recreational areas; therefore, non-occupational dermal exposure and risk to adults, infants and children are low. Because the use sites are agricultural, exposure to infants and children in school, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to infants and children is expected to be negligible.

3.3 Dietary Exposure and Risk Assessment

3.3.1 Food

As there are no direct applications to food, there is no concern for risks posed by dietary exposure of the general population, including infants and children, or animals. Consequently, the establishment of an MRL is not required for *T. virens* strain G-41. As well, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Consequently dietary risks are minimal to non-existent.

3.3.2 Drinking Water

The likelihood that *Trichoderma virens* strain G-41 could enter neighbouring aquatic environments as a result of the use of RootShield Plus Wettable Powder in potting mix and soil of outdoor container-grown ornamentals is negligible. No risks are expected from exposure to this microorganism via drinking water because exposure will be minimal and because there were no harmful effects observed in Tier I acute oral toxicity and infectivity testing. The RootShield Plus Wettable Powder label instructs users not to contaminate irrigation or drinking water supplies or aquatic habitats through equipment cleaning or waste disposal. Users are also prohibited from allowing runoff from the application of this product to enter lakes, streams, ponds or other waters. Furthermore, municipal treatment of drinking water is expected to remove any residues transferred to drinking water. Therefore, potential exposure to *Trichoderma virens* strain G-41 in surface and drinking water is negligible.

3.3.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

As the end-use product, RootShield Plus Wettable Powder, is not approved for direct applications to food crops, dietary risks to sensitive subpopulations is not expected for *T. virens* strain G-41.

3.4 Maximum Residue Limits

As there are no direct applications to food and no significant adverse effects were reported in the Tier I acute toxicity/pathogenicity studies, a maximum residue limit (MRL) is not required for *Trichoderma virens* strain G-41.

3.5 Aggregate Exposure

As dietary (food and drinking water) exposures and non-occupational exposures (dermal and inhalation) are expected to be minimal to non-existent, an aggregate exposure assessment was not conducted for *T. virens* strain G-41.

3.6 Cumulative Effects

The PMRA has considered available information on the cumulative effects of such residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity.

Besides naturally occurring strains of *Trichoderma virens* in the environment, the PMRA is not aware of any other microorganisms, or other substances that share a common mechanism of toxicity with this active ingredient. No cumulative effects are anticipated if the residues of *Trichoderma virens* strain G-41 interact with related strains of this microbial species.

4.0 Incidents Reports

Since April 26, 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the Pesticides and Pest Management portion of Health Canada's website http://www.healthcanada.gc.ca/pesticideincident. Only incidents in which the pesticide is determined to be linked to the effects (Canadian causality of highly probable, probable and possible; U.S. causality of highly probable, probable and possible) are considered in the reviews.

As of September 23, 2011, there have been no incidents related to health reported to the PMRA, nor summarized by the U.S. EPA or the California Department of Pesticide regulation (CalDPR), for products containing *Trichoderma virens* strain G-41.

As of December 7, 2011, there were no environmental incidents reported in the PMRA Incident reporting database nor in the U.S. EPA's Ecological Incident Information System (EIIS) for products containing *T. virens* strain G-41 for use as pesticides.

5.0 Impact on the Environment

5.1 Fate and Behaviour in the Environment

Environmental fate testing is intended to demonstrate whether a microbial pest control agent (MPCA) is capable of surviving or replicating in the environment to which it is applied, and could provide an indication of which non–target organisms may be exposed to the MPCA as well as an indication of the extent of exposure. Environmental fate data are not normally required for Tier I risk assessment purposes and are only triggered if significant toxicological effects in non–target organisms are noted in Tier I testing. Since no significant toxicological effects in non–target organisms are expected from the proposed use of RootShield Plus Wettable Powder to treat the growing media of outdoor container-grown ornamentals, no fate data are required to complete the environmental risk assessment of G-41 Technical and RootShield Plus Wettable Powder.

Trichoderma spp. are common soil hyphomycetes found in all climate zones ranging from Antarctica to the tropics. *Trichoderma virens* strain G-41 is naturally occurring in North America, and can be found in soils and on decaying wood and vegetable matter but is not expected to proliferate in aquatic environments. *Trichoderma* spp. have been detected in various types of soil at concentrations ranging from 10^4 to 10^6 colony forming units (CFU)/g. Based on the maximum application rate of RootShield Plus Wettable Powder, the estimated amount of MPCA applied to the soil surface is approximately 6.7×10^3 CFU/cm^2 of soil. Therefore, outdoor directed applications to soil are not expected to result in soil concentrations that are substantially above normal concentrations, and exposure in marine or estuarine environments resulting from runoff is expected to be similar to that which would occur as a result of natural soil concentrations of *Trichoderma* species.

Trichoderma virens strain G-41 is a known producer of gliotoxin a secondary metabolite of toxicological significance. The technical and end-use products are expected to contain gliotoxin. There is no known production of any other genotoxic, carcinogenic, allergenic, mutagenic or toxic metabolites by *T. virens* strain G-41.

5.2 Effects on Non-Target Species

Acceptable waiver rationales were used to address the environmental toxicology requirements. These rationales were based on the ubiquitous nature of *T. virens* strain G-41, the lack of adverse effects reported in the literature, and the inability of *T. virens* strain G-41 to become established in aquatic environments.

5.2.1 Effects on Terrestrial Organisms

The data and information submitted or available on the proposed microbial pest control agent (MPCA) plants and use pattern are sufficient to adequately characterize the risk to the terrestrial environment from the use of the end-use product RootShield Plus Wettable Powder.

The assessment of risk was based on waiver rationales submitted in lieu of conducting product specific testing. Requests to waive environmental toxicity testing for wild mammals, avian oral and inhalation, terrestrial plants, honey bees and other terrestrial arthropods were essentially based on lack of effects toward these non-target organisms in the published scientific literature, given the ubiquitous nature of *Trichoderma* spp. in the environment.

An acceptable rationale to waive the data for wild mammals and birds was based on the common occurrence of *Trichoderma* spp. in the environment and the high likelihood that these non-target organisms are commonly exposed with apparent lack of adverse effects as supported by literature searches. In addition *T. virens* strain G-41 is not expected to grow well at avian and mammalian body temperatures and laboratory studies showed that the MPCA is not toxic or pathogenic in rats via the IV route (10^7 CFU per animal). Finally it is toxicologically similar to *Trichoderma (Gliocladium) virens* strain GL-21, which was previously determined by the U.S. EPA to present minimal risk to wild mammals and birds.

An acceptable rationale to waive the data for honey bees and other non-target terrestrial arthropods was based on strain G-41's natural occurrence in soil and that it is not recognized as an entomopathogen and does not cause insect epizootics. While honey bees may come in contact soil treated with *T. virens* strain G-41, they primarily associate with foliar surfaces of plants. Therefore, since RootShield Plus Wettable Powder is intended for soil directed use only, exposure to honey bees will likely be limited. Furthermore the inert ingredients/formulants are not expected to contribute to potential toxicity. Based on the proposed soil treatment of potting media intended for outdoor ornamentals, adverse effects to honey bees and other non-target terrestrial arthropods are expected to be minimal.

A literature search for possible effects on insects and other invertebrates in the Environmental Information Database available to EPA was performed for *Trichoderma virens*. This database simultaneously searches six databases (Agricola, Biosis Previews, CAB Abstracts, Energy Science and Technology, General Science Abstracts, and the National Technical Information Service) for the years 1926 - present. The term "*Trichoderma*" was paired with each of the terms, "insect," "arthropod," and "entomopathogen" to perform the searches. Since *Trichoderma* spp. are common in the soil, some reports were available showing associations of these fungi with insect species (often as normal microflora); however, no reports of insect toxicity or pathogenicity attributed to *T. virens* were found in this search. *Trichoderma virens* is not widely recognized as an entomopathogen, and is not widely known to cause insect epizootics. Therefore, adverse effects to non-target insects as a result of the proposed applications of *T. virens* strain G-41 are not expected. This scientific rationale was classified as acceptable and satisfies the data requirement for terrestrial arthropods (including honey bees) testing.

An acceptable rationale to waive the data for terrestrial non-arthropods was based on *T. virens* strain G-41's natural occurrence in soil and that it is not recognized as an annelid or mollusc pathogen. Based on lack of adverse effects as supported by the scientific literature the risk to non-target terrestrial non-arthropods is expected to be minimal.

An acceptable rationale to waive the data for non-target terrestrial plants was based on the natural occurrence of *T. virens* strain G-41 in soil and that plants are commonly exposed to the MPCA with an apparent lack of adverse effects as supported by the scientific literature. In addition, the proposed use of RootShield Plus Wettable Powder is expected to result in concentrations of the MPCA that are similar to natural levels in the environment. Therefore, since *T. virens* strain G-41 is a common soil fungus and is not related to any known plant pathogens, adverse effects to terrestrial plants are not expected to result from the proposed use as a soil treatment for outdoor container-grown ornamentals.

Gliotoxin, which is expected to be present in the EP has been found to have phytotoxic effects on some plant species, but effects on plants were also found to be strain-specific. Based on the proposed use pattern, exposure to non-target terrestrial plants is expected to be minimal.

A request to waive the requirement for microorganisms was not submitted. However *T. virens*, in general, has not been found to be active against other beneficial fungi such as mycorrhizal species of *Glomus*. No effects of *T. virens* strain G-41 on fungal species other than those listed as target species have been reported in the published scientific literature.

The other active ingredient in RootShield Plus Wettable Powder is *T. harzianum* strain KRL-AG2 and aggressive strains of *T. harzianum* were identified in published literature as the cause of "green mold disease" in the commercial mushroom industries in Europe and North America. A concern was therefore identified from the use of plant material treated with RootShield Plus Wettable Powder, as a growing substrate in mushroom houses. As a result a mitigative statement appears on the label prohibiting the use of treated material in mushroom growing facilities. Given that *Trichoderma* species are ubiquitous and considering the above information, the risk to other beneficial microorganisms is considered to be low from the proposed outdoor use pattern of RootShield Plus Wettable Powder.

The proposed soil/growing media treatment use is not expected to result in a sustained increase in the background levels of *T. virens* strain G-41 in the terrestrial environment. In summary there is no known relationship reported in the published scientific literature between *T. virens* strain G-41 and known terrestrial animal pathogens and the risk posed by the proposed use of RootShield Plus Wettable Powder as a soil treatment for outdoor container-grown ornamentals is not expected to present a concern for mammals, birds, terrestrial invertebrates, microorganisms or plants.

5.2.2 Effects on Aquatic Organisms

The data and information submitted or available on the proposed microbial pest control agent (MPCA) and use pattern are sufficient to adequately characterize the risk to the aquatic environment from the use of the end-use product RootShield Plus Wettable Powder.

The assessment of risk was based on waiver rationales submitted in lieu of conducting product specific testing. Requests to waive environmental toxicity testing for fishes, aquatic invertebrates and aquatic plants were based on the low likelihood for exposure to the aquatic environment from the proposed use pattern and a lack of effects toward these non-target organisms in the published scientific literature.

An acceptable rationale to waive the data for freshwater or marine fishes was based on the lack of adverse effects anticipated from exposure to *T. virens* strain G-41 as supported by a literature search. In addition the MPCA is a soil microorganism and is not expected to proliferate in aquatic environments; following application, concentrations of the MPCA are expected to be similar to natural levels common in the environment.

A literature search for possible effects on fish was performed for *T. virens* in the Environmental Information Database available to EPA. This database simultaneously searches six databases (Agricola, Biosis Previews, CAB Abstracts, Energy Science and Technology, General Science Abstracts, and the National Technical Information Service) for the years 1926 - present. The term "*Trichoderma*" was paired with each of the terms, "aquatic," "freshwater," "marine" and "fish," to perform the searches. Several reports of *Trichoderma* spp. isolated from aquatic environments were found; therefore, sufficient information is not available to confirm that *T. virens* strain G-41 would not survive in water but it is not expected to proliferate. Furthermore, reports of toxic or pathogenic effects in fish caused by *T. virens* were not found in this search. Thus, adverse effects to freshwater or marine fishes are not expected.

An acceptable rationale to waive the data for freshwater or marine invertebrates was based on the lack of adverse effects anticipated from exposure to *T. virens* strain G-41 as the MPCA is a soil microorganism and is not expected to proliferate in aquatic environments. Following application concentrations of the MPCA are expected to be similar to natural levels common in the environment, aquatic invertebrates are commonly exposed to this fungus and the formulants in G-41 Technical are not expected to contribute to potential toxicity. The end-use product is, however, expected to contain gliotoxin a metabolite of toxicological significance.

Published ecotoxicity values for gliotoxin include a 36-hour LC_{50} value of 0.16 ppm for *Daphnia magna* (OECD guideline 202) and a 36-hour LC_{50} value of 6.66 ppm for *Artemia salina*. The reported 24-hour LC_{50} values for *D. magna* and *A. salina* were 0.28 ppm, and 12.87 ppm, respectively. However, exposure of freshwater or marine invertebrates to *T. virens* strain G-41 is not expected to be greater than exposure to naturally occurring levels of *T. virens* and based on the lack of adverse effects in the scientific literature, adverse effects to freshwater or marine invertebrates are not expected.

An acceptable rationale to waive data for effects to aquatic non-target plants was based on the low likelihood for exposure from run-off and lack of adverse effects toward these plants in the published scientific literature, given the ubiquitous nature of *T. virens* strain G-41 in the environment. Exposure of aquatic plants to *T. virens* strain G-41 is not expected to be greater than exposure to naturally occurring levels of *T. virens*. Thus, adverse effects to aquatic plants are not expected.

The end-use product containing *T. virens* strain G-41 is intended for soil directed use only and applications to soil are not expected to result in soil concentrations that are substantially above normal concentrations, and exposure in aquatic environments resulting from runoff is expected to be similar to that which would occur as a result of natural soil concentrations of *Trichoderma* species. Furthermore, there is no known relationship reported in the published scientific literature between *T. virens* strain G-41 and known aquatic animal pathogens and the risk posed by the proposed use of RootShield Plus Wettable Powder is not expected to present a concern for fishes, and aquatic insects and plants.

6.0 Value

6.1 Effectiveness Against Pests

6.1.1 Acceptable Efficacy Claims

A total of 14 greenhouse trials were submitted for review. The data submitted measured the effect of treatments on root and shoot weights, and plant height and provided visual assessments of comparative root growth and plant aesthetics. No direct assessments of root disease incidence or severity were made. Results from the submitted trials showed numerical or statistically significant increases in root weights, shoot weights and/or plant heights in treated plants implying reduced root rot.

The claim of suppression of root rot caused by *Phytophthora cinnamomi* was supported on outdoor container-grown ornamentals based on the submitted data. The claim of suppression of root rot caused by *P. parasitica* was supported on outdoor container-grown ornamentals; additional data has been requested to confirm efficacy.

6.2 Economics

The applicant provided economic information on the ornamental industry in Canada. The following is a summary of that information.

A report sponsored by the Canadian Ornamental Horticulture Alliance published in January 2009 discussed the economic importance of ornamentals in Canada. In 2006, horticulture sales were $5.4 billion with 41.8% of those sales occurring in the ornamental sector. The annual growth rate in this sector is approximately 1.7%.

Root rot in ornamentals affects the aesthetic quality of plants. In the 2003 report to Ontario Horticultural Crops Research and Services Committee, the Ontario Greenhouse and Protected Crops Research and Services Sub-committee stated that industry concerns included lack of sufficient registrations of IPM-compatible products. Registration of RootShield Plus Wettable Powder will provide growers with an effective treatment for root rot on outdoor container-grown ornamentals which can be integrated into an IPM program.

6.3 Sustainability

6.3.1 Survey of Alternatives

Alternative products are available for the disease claims on outdoor container-grown ornamentals. For additional information on alternative products, see Appendix 1, Table 3.

6.3.2 Compatibility with Current Management Practices Including Integrated Pest Management

IPM for control of root pests on outdoor container-grown ornamentals includes monitoring for pests, good sanitization (crop material, soil, structures) and environmental management (irrigation, fertilization, light, air flow, drainage). The use of resistant cultivars is recommended when available. As a potting mix incorporated or drench applied product, RootShield Plus Wettable Powder can be added to an IPM program including cultural methods. The use of RootShield Plus Wettable Powder was not demonstrated in alternation with other fungicides.

6.3.3 Contribution to Risk Reduction and Sustainability

RootShield Plus Wettable Powder contains two naturally occurring soil fungi with multiple modes of action, including competitive exclusion, production of inhibitory compounds and induction of plant defences. The risk of plant pathogens developing resistance to this product is low.

7.0 Pest Control Product Policy Considerations

7.1 Toxic Substances Management Policy Considerations

The Toxic Substances Management Policy (TSMP) is a federal government policy developed to provide direction on the management of substances of concern that are released into the environment. The TSMP calls for the virtual elimination of Track 1 substances [those that meet all four criteria outlined in the policy, in other words persistent (in air, soil, water and/or sediment), bio-accumulative, primarily a result of human activity and toxic as defined by the *Canadian Environmental Protection Act*].

G-41 Technical and RootShield Plus Wettable Powder were assessed in accordance with the PMRA Regulatory Directive DIR99-031

- G-41 Technical does not meet the Track 1 criteria because the active ingredient is a biological organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products.
- There are also no formulants, contaminants or impurities present in the end-use product that would meet the TSMP Track-1 criteria.

7.2 Formulants of Health or Environmental Concern

During the review process, contaminants in the technical and formulants and contaminants in the end-use products are compared against the *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* maintained in the *Canada Gazette*[2]. The list is used as described in the PMRA Notice of Intent NOI2005-01[3] and is based on existing policies and regulations including: DIR99-03; and DIR2006-02[4] and taking into consideration the Ozone-depleting Substance Regulations, 1998, of the *Canadian Environmental Protection Act* (substances designated under the Montreal Protocol). The PMRA has reached the following conclusions:

- The TGAI, G-41 Technical, contains wheat which is identified in the *Canada Gazette*, Part II, Volume 139, Number 24, pages 2641-2643: *List of Pest Control Product Formulants of Health or Environmental Concern* as allergens known to cause anaphylactic-type reactions. Therefore, the label for both G41 Technical and RootShield Plus Wettable Powder will include the warning statement "Warning: this product contains the allergen wheat" on the principal display panel.

- The TGAI, G-41 Technical does not contain any contaminants of environmental concern identified in Canada Gazette Part II, Volume 139, Number 24, pages 2641–2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern*.

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1 Regulatory Directive DIR99-03, The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy

2 *Canada Gazette*, Part II, Volume 139, Number 24, SI/2005-11-30) pages 2641-2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* and in the order amending this list in the *Canada Gazette*, Part II, Volume 142, Number 13, SI/2008-67 (2008-06-25) pages 1611-1613: *Part 1 Formulants of Health or Environmental Concern, Part 2 Formulants of Health or Environmental Concern that are Allergens Known to Cause Anaphylactic-Type Reactions and Part 3 Contaminants of Health or Environmental Concern.*

3 Notice of Intent NOI2005-01, List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern under the New Pest Control Products Act

4 Regulatory Directive DIR2006-02, *PMRA Formulants Policy*

- The end-use product, RootShield Plus Wettable Powder, contains a formulant which is a mixture containing sulphites. Sulphites are identified in the *Canada Gazette,* Part II, Volume 139, Number 24, pages 2641-2643: *List of Pest Control Product Formulants of Health or Environmental Concern* as allergens known to cause anaphylactic-type reactions. Therefore, the label for RootShield Plus Wettable Powder will include the precautionary statement "Warning: this product contains the allergen sulphites" on the principal display panel.

- The end-use product, RootShield Plus Wettable Powder does not contain any formulants or contaminants of environmental concern identified in Canada Gazette Part II, Volume 139, Number 24, pages 2641–2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern*.

The use of formulants in registered pest control products is assessed on an ongoing basis through PMRA formulant initiatives and DIR2006–02.

8.0 Summary

8.1 Methods for Analysis of the Micro-organism as Manufactured

The product characterization data for G-41 Technical and RootShield Plus Wettable Powder were judged to be adequate to assess their potential human health and environmental risks. The technical material was fully characterized and the specifications were supported by the analyses of a sufficient number of batches. Storage stability data submitted were sufficient to support a shelf life of 6 months at up to 23°C.

8.2 Human Health and Safety

The acute toxicity and infectivity studies submitted in support of *Trichoderma virens* strain G-41 were determined to be sufficiently complete to permit a decision on registration. *Trichoderma virens* strain G-41 was of low toxicity in the rat when administered via oral, inhalation, and dermal routes and was not infective via the intraperitoneal routes of exposure. RootShield Plus Wettable Powder is expected to cause mild irritation to the eye but is not irritating to the skin.

Trichoderma virens strain GL-21, a toxicologically similar strain to the MPCA, was toxic, but not infective, via the intratracheal route. Intravenous injection of *Trichoderma virens* strain GL-21 was also toxic but not infective.

A request to waive acute pulmonary toxicity/pathogenicity testing with the G-41 Technical was accepted based on a lack of toxicity of the end-use product via the inhalation route, and of the TGAI via the intraperitoneal route, and the lack of toxicity and pathogenicity of *Trichoderma virens* strain GL-21 (a toxicologically similar strain) via the intratracheal route.

When used according to the label instructions, the potential for dermal, pulmonary and ocular exposure for persons mixing and loading RootShield Plus Wettable Powder exists, with the primary source of exposure to workers being dermal and, to a lesser extent, pulmonary. Precautionary statements on product labels and the wearing of personal protective equipment (PPE), including a dust/mist filtering respirator/mask and protective eye wear, will adequately mitigate the risks from exposure.

While *Trichoderma virens* strain G-41 has the potential to be a sensitizing agent, inhalation and dermal exposure is not a concern if the required dust/mist filtering respirator/mask and appropriate PPE stipulated on the end-use product label is worn by handlers and applicators of RootShield Plus Wettable Powder. Furthermore, precautionary labelling will alert users of the potential sensitization hazard of the end-use products.

Occupational exposure to gliotoxin is also not expected to pose a health concern based on the requirement for personal protective equipment, particularly the dust/mist filtering respirator for biological products and the instructions to avoid skin contact with treated soil or potting mix.

The health risk to the general population, including infants and children, as a result of bystander exposure and/or chronic dietary exposure is expected to be minimal since RootShield Plus Wettable Powder will only be applied to container-grown outdoor ornamentals. The product is not to be applied to residential or recreational areas or to food or feed crops.

8.3 Environmental Risk

The scientific rationales and supporting published scientific literature submitted in support of *T. virens* strain G-41 were determined to be sufficiently complete to permit a decision on registration. The use of RootShield Plus Wettable Powder containing *T. virens* strain G-41 is not expected to pose a risk to non-target organisms including birds, mammals, arthropods, fish, and plants.

No additional studies were required to address the environmental fate and behaviour of *T.* virens strain G-41. Environmental fate data are higher tier requirements and are not normally required in the absence of significant toxicological effects in non-target organisms in Tier I testing. Environmental exposure to *T. virens* strain G-41 is expected to be minimal given the proposed use of RootShield Plus Wettable Powder as a treatment of potting media for outdoor container-grown ornamentals.

As a general precaution, the RootShield Plus Wettable Powder label prohibits its direct application to aquatic habitats (such as lakes, streams and ponds).The label also directs users to avoid contaminating surface water by disposal of equipment wash waters.

8.4 Value

The submitted data demonstrates increases in root and shoot weights and plant height as a result of treatment with RootShield Plus Wettable Powder. These increases suggest that the product is suppressing root rots caused by *Phytophthora cinnamomi* and *Phytophthora parasitica* on outdoor container-grown ornamentals. Morphometric data suggests that RootShield Plus Wettable Powder is suppressing *Phytophthora* root pests; additional data reporting direct assessments of disease incidence and severity has been requested to confirm the results.

Few conventional fungicides are registered to control the proposed diseases on outdoor container-grown ornamentals. Two other biological products are registered on these crops, both of which suppress the proposed diseases, as well as two conventional products registered for control. The registration of RootShield Plus Wettable Powder will provide another option to growers for disease management.

9.0 Proposed Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of G-41 Technical and RootShield Plus Wettable Powder, containing the technical grade active ingredients *Trichoderma virens* strain G-41 and *Trichoderma hazianum* Rifai strain KRL-AG2, to suppress certain *Phytophthora* species that cause root rot on outdoor container-grown ornamentals.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

List of Abbreviations

bw	body weight
CFU	colony forming unit
cm	centimetre(s)
DACO	data code
EP	end-use product
FDA	*Food and Drugs Act*
g	gram
HPLC	high pressure liquid chromatography
IP	intraperitoneal
IPM	Integrated Pest Management
IV	intravenous
kg	kilogram
L	litre
LC_{50}	lethal concentration 50%
LD_{50}	lethal dose 50%
m	metre(s)
MAS	mean average score
mg	milligram
MIS	maximum irritation score
mL	millilitre
MPCA	microbial pest control agent
MRL	maximum residue limit
MSHA	Mine Safety and Health Administration
NIOSH	The National Institute for Occupational Safety and Health
OECD	Organisation for Economic Co-operation and Development
PDA	potato dextrose agar
PMRA	Pest Management Regulatory Agency
PPE	personal protective equipment
ppm	parts per million
TGAI	technical grade active ingredient
TSA	tryptic soy agar
TSMP	Toxic Substances Management Policy
wt	weight
U.S.EPA	United States Environmental Protection Agency

Appendix I Tables and Figures

Table 1 Toxicity and Infectivity of *Trichoderma virens* strain G-41 and its associated end-use product (RootShield Plus Wettable Powder)

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *Trichoderma virens* strain G-41				
Acute Oral Toxicity/ Pathogenicity	Rat-Sprague Dawley 12/sex, $>10^8$ CFU of G-41 Technical in phosphate buffered saline per animal; 0.1 mL dose Interim sacrifices (3/sex) on Day 3, 7, and 14 Untreated control group: 5/sex Shelf control group: 2/sex Inactivated test substance: 5/sex 21-day observation period	LD_{50} (male; female) $>10^8$ CFU/animal	No mortalities, no clinical signs of treatment related toxicity. Body weight was largely unaffected. No necropsy findings other than an empty digestive system in one animal from the test substance group. The test substance was detected in the fecal samples on Day 1. Counts were below clearance threshold in the brain, lungs, liver, spleen, kidneys, and lymph by Day 3, and by Day 7 for the cecum contents. Pathogenicity not unequivocally assessed. (low sensitivity of detection; <10%). **LOW TOXICITY ACCEPTABLE**	PMRA 2003619

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *Trichoderma virens* strain G-41				
Acute Oral Toxicity/ Pathogenicity	Rat-Sprague Dawley 13/sex, $>10^8$ CFU GL-21 in 2.0% carbylmethyl cellulose; interim sacrifices (3/sex) on Day 1, 7, and 14 Untreated control group: 13/sex; 14-day observation period	LD_{50} (male; female) $>10^8$ CFU/animal	No mortalities or effect on body weight gain. General signs of toxicity (lethargy, rapid breathing) were resolved by Day1. No gross findings observed at necropsy. The test substance was initially detected in the feces (Day 1) and lungs (Day 7), but had cleared from all tissues/organs by Day 14. **LOW TOXICITY, NOT INFECTIVE** **ACCEPTABLE but supplemental**	PMRA 1979843

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *Trichoderma virens* strain G-41				
Acute Pulmonary Toxicity and Infectivity	Rat-Sprague Dawley 15/sex, 10^8 CFU of *T. virens* strain GL-21 in saline/animal; 0.04 mL dosing volume Interim sacrifices on Day 1, 7, and 14. Untreated control group: 15/sex; 21-day observation period	LD_{50} (male; female) $> 10^8$ CFU/animal	No mortalities. Body weight was largely unaffected. General signs of toxicity (poor weight gain; lethargy; rapid breathing; rough hair coat) observed in all animals after dosing, but resolved by Day 4 or sooner. The test substance was detected in organs/tissues on Day 1, but had cleared from all tissues/organs by Day 7, except for the spleen and lungs which had low counts until Day 14, and Day 21, respectively. A pattern of clearance was established. Necropsy revealed grey spots in the lungs, enlarged/pale peritracheal lymph nodes at each interim sacrifice. Toxicity/necropsy findings are attributed to administration of bolus material **LOW TOXICITY, NON-INFECTIVE** **ACCEPTABLE but supplemental**	PMRA 1979846
Acute Pulmonary Toxicity and Infectivity	Waiver submitted. The waiver request is based on a lack of toxicity of RootShield Plus Wettable Powder via the inhalation route (PMRA 1807271), and a lack of toxicity *T. virens* strain G-41 via the intraperitoneal route (PMRA 1986053). Furthermore, *T. virens* strain GL-21, a toxicologically similar strain to *T. virens* strain G-41, demonstrated a lack of toxicity and pathogenicity via the intratracheal route (PMRA 1979846). **WAIVER ACCEPTED**			PMRA 1989042 and PMRA 1986046

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *Trichoderma virens* strain G-41				
Intravenous Injection Infectivity	Rat-Sprague Dawley 21/sex, 0.1 g of mycelia of GL-21 in saline/animal; 0.5 mL dosing volume Untreated control group: 15/sex Interim sacrifices: Day 1, 7, 14 and 21 21-day observation period	LD_{50} (male; female) > 0.1g mycelia of GL-21/animal	8/30 animals died by Day 2. Toxicity (lethargy, rough hair coat, hunched posture, rapid breathing) observed in all surviving animals until Day 8. Gross necropsy (2 animals from Day 1) showed discoloured lungs (slightly red-dark red), stiffened heart, and a fluid-filled thoracic cavity (1 animal). Body weight gain was largely unaffected. The test organism was detected in tissues/organs on Day 0, and had cleared from all organs (except the spleen) by Day 14 or sooner. By Day 21, clearance from the spleen was also achieved. The test organism was not detected in the blood at any time point. Toxicity/necropsy findings were attributed to mechanical damage (in other words, clogging of capillaries in lungs) from injection of the cell mass rather. **TOXIC, NOT INFECTIVE** **ACCEPTABLE but supplemental**	PMRA 1979848

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *Trichoderma virens* strain G-41				
Intraperitoneal Injection Infectivity	Rat-Wistar 3/sex, 10^7 CFU G-41 Technical distilled water /animal; 1 mL dosing volume Vehicle control group: 3/sex 21-day observation period	LD_{50} (male; female) > 10^7 CFU/animal	No mortalities, no clinical signs of toxicity, no gross abnormalities observed in any of the test animals. Body weight gain was largely unaffected. **LOW TOXICITY, NOT INFECTIVE** **ACCEPTABLE**	PMRA 1986053
Acute Toxicity/Infectivity of RootShield Plus Wettable Powder[2]				
Acute Oral Toxicity	Rat-Sprague Dawley; 3 female, 5000 mg of RootShield Plus Wettable Powder in deionized water/kg bw (potency not reported) 14-day observation period	LD_{50} (female) > 5000 mg/kg bw	No mortalities or effect on body weight gain and no clinical signs of toxicity. No gross findings observed at necropsy. **LOW TOXICITY** **SUPPLEMENTAL**	PMRA 1807270
Acute Inhalation Toxicity	Rat-Sprague Dawley 5/sex, 5.14 mg of RootShield Plus Wettable Powder /L (potency not reported), 4-hour nose-only exposure 14-day observation period	LC_{50} (male; female) > 5.14 mg/L	No mortalities and no effect on body weight gain. Clinical signs of general toxicity (decreased activity; piloerection) were resolved by Day 2. **LOW TOXICITY** **SUPPLEMENTAL**	PMRA 1807271
Acute Dermal Toxicity	Rat-Sprague Dawley, 5/sex: 5050 mg of RootShield Plus Wettable Powder suspended in deionized water/kg bw 14-day observation period Irritation scored by Draize (1944)	LD_{50} (male; female) >5050 mg	No mortalities, no treatment-related clinical signs, no necropsy findings and no major changes in body weight. No signs of dermal toxicity or irritation at any time point. **LOW TOXIC NON-IRRITATING** **ACCEPTABLE**	PMRA 1807268

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *Trichoderma virens* strain G-41				
Acute Dermal Irritation	Rabbit-New Zealand White, 2 male; 1 female: 500 mg of RootShield Plus Wettable Powder (potency not measured) 4-hour exposure 72-hour observation period Irritation scored by Driaze (1944)	MAS (male; female)= 0	No signs of dermal irritation at any time point. **NON-IRRITATING** **ACCEPTABLE**	PMRA 1807269
Eye Irritation	Rabbit- New Zealand White (1 male; 2 female): 57.3g of RootShield Plus Wettable Powder in 0.1 mL deionized water into one eye of each rabbit (potency not reported) 7-day observation period.	MIS= 18.0 MAS= 13.78 (effects cleared by Day 4)	Corneal opacity with redness, chemosis and discharge of the conjunctivae were observed in all eyes at 24-hours. Redness and chemosis persisted until 72-hours. All effects had cleared by Day 4. Based on the MAS of 13.78 and considering the persistence of ocular irritation (72-hours), RootShield Plus Wettable Powder is mildly irritating to the eyes. **MILD EYE IRRITANT** **ACCEPTABLE**	PMRA 1807272
Hypersensitivity testing	Waiver submitted. The waiver request was based on the lack of incidents of hypersensitivity or other adverse effects throughout the research of G-41 Technical, as well as the natural occurrence of the MPCA as a ubiquitous soil fungus and the nature of the inert ingredients in the TGAI. **WAIVER ACCEPTED**			PMRA 1986060 and PMRA 1986061

[1] A comparative study indicated that *T. virens* strain GL-21 is toxicologically similar to the MPCA (*T. virens* strain G-41)
[2] The test substance RootShield Plus Wettable Powder contained 0.60% *T. virens* strain G-41 and 1.15% *T. harzianum* strain KRL-AG2, rather 9.5% and 5.0%, respectively as per the proposed formulation of RootShield Plus Wettable Powder. Potency not provided.
MAS= Mean Average Score; MIS= Maximum Irritation Score

Table 2 Toxicity to Non-Target Species

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Terrestrial Organisms				
Vertebrates				
Birds	A request to waive the requirement for test data was submitted based on the properties of the MPCA and the limited potential for exposure to birds from the proposed use of RootShield Plus Wettable Powder as a soil treatment. No adverse effects to birds were found in the published scientific literature. In addition, *Trichoderma virens* G-41 is not expected to grow at normal bird body temperatures. **WAIVER RATIONALE ACCEPTED**			PMRA 1986099
	Pulmonary	A request to waive the requirement for test data was submitted based on the properties of the MPCA and the limited potential for pulmonary exposure to avian species from the proposed use of RootShield Plus Wettable Powder No adverse effects to birds were reported in the published scientific literature. **WAIVER RATIONALE ACCEPTED**		PMRA 1986111
Wild Mammals	A request to waive the requirement for test data was submitted based on the properties of the MPCA and the limited potential for exposure to wild mammals from the proposed use of RootShield Plus Wettable Powder as a soil treatment. No adverse effects to mammals were found in the published scientific literature. In addition, no pathogenicity was observed in acute mammalian toxicity and infectivity testing . **WAIVER RATIONALE ACCEPTED**			PMRA 1986124
Invertebrates				
Arthropods				
Terrestrial Arthropods	Honey bee (*Apis mellifera*)	A request to waive the requirement for test data was also submitted based on the properties of the MPCA and the limited potential for exposure to terrestrial arthropods from the proposed use of RootShield Plus Wettable Powder as a soil treatment.		PMRA 1986134
	A request to waive the requirement for test data was also submitted based on the properties of the MPCA and the limited potential for exposure to terrestrial arthropods from the proposed use of RootShield Plus Wettable Powder as a soil treatment. Adverse effects to non-target insects resulting from the proposed applications of *T. virens* strain G-41 are not expected based on it's natural occurrence in soil and the fact that it is not recognized as an entomopathogen and does not cause insect epizootics. **WAIVER RATIONALE ACCEPTED**			PMRA 1986134
Non-arthropods				
Terrestrial Non-Arthropod Invertebrates	A request to waive the requirement for these test data was based on strain G-41's natural occurrence in soil and the fact that it is not recognized as an annelid or mollusc pathogen, **WAIVER RATIONALE ACCEPTED**			PMRA 1986149

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Microorganisms				
	A request to waive the requirement for test data was not submitted. However given the ubiquitous nature of the MPCA and that its use outdoors is not expected to result in sustained populations of *T. virens* strain G-41 above background levels, adverse effects to non–target microorganisms is not expected. Therefore testing has been waived.			
Plants				
Plants	A request to waive the requirement for actual test data was submitted based on the properties of the MPCA and the limited potential for exposure to non-target terrestrial plants from the proposed use of RootShield Plus Wettable Powder. *Trichoderma virens* strain G-41 is not a plant pathogen and no adverse effects to terrestrial plants were reported in the published scientific literature. **WAIVER RATIONALE ACCEPTED**			PMRA 1986153 1986156
Aquatic Organisms				
Vertebrates				
Fish	A request to waive the requirement for actual test data was submitted based on the properties of the MPCA and the limited potential for exposure to fish from the proposed use of RootShield Plus Wettable Powder. *Trichoderma virens* strain G-41 is not a fish pathogen and no adverse effects to freshwater and marine fishes were found in the published scientific literature. **WAIVER RATIONALE ACCEPTED**			PMRA 1986130 1986127
Invertebrates				
Aquatic Arthropods and Non-Arthropod Invertebrates	A request to waive the requirement for actual test data was submitted based on the properties of the MPCA and the limited potential for exposure to the aquatic environment from the proposed use of RootShield Plus Wettable Powder as a soil treatment. No adverse effects to freshwater and marine invertebrates were found in the published scientific literature. **WAIVER RATIONALE ACCEPTED**			PMRA 1986138 1986142
Plants				
Aquatic Plants	A request to waive the requirement for actual test data was submitted based on the properties of the MPCA and the limited potential for exposure to aquatic plants from the proposed use of RootShield Plus Wettable Powder as a soil treatment. *Trichoderma virens* strain G-41 is not a plant pathogen and no adverse effects to aquatic plants were found in the published scientific literature. **WAIVER RATIONALE ACCEPTED**			PMRA 1986155 1986156

Table 3 Alternative Active Ingredients Registered for the Management of Root Rot caused by the Pathogens Supported on the RootShield Plus Wettable Powder label

Crop	Pest	Active Ingredient	Mode of Action Group	Level of Control
Outdoor Container-Grown Ornamentals	*Phytophthora cinnamomi, P. parasitica*	*S. griseoviridis* strain K61 (greenhouse)	N/A	Suppression
		Bacillus subtilis strain QST 713	44	Suppression
		fosetyl-AL	33	Control
		propamocarb hydrochloride	28	Control
		fluopicolide	43	Control

Table 4 Use (label) Claims Proposed by Applicant and Whether Acceptable or Unsupported

Proposed use claim	Supported / Unsupported
Suppression of root rot caused by *Phytophthora cinnamomi* on container-grown ornamentals	Supported.
Suppression of root rot caused by *Phytophthora parasitica* on container-grown ornamentals	Supported. Confirmatory efficacy data have been requested.

References

A. List of Studies/Information Submitted by Registrant

1.0 Methods of Analysis

PMRA No. **Title**

1986028 2009. Product Chemistry of G-41 Technical. DACO: M2.10.3,M2.11,M2.12,M2.7.1, M2.7.2,M2.8,M2.9, M2.9.1,M2.9.2,M2.9.3 CBI

1986030 2010, Supplemental Product Chemistry of G-41 Technical, DACO: M2.10,M2.7.2, M2.8,M2.9.1 CBI

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1986076 Flores, A., I. Chet and A. Herrera-Estrella. 1997. Improved biocontrol activity of *Trichoderma harzianum* by over-expression of the proteinase-encoding gene prb1.Curr. Genet. 31: 30-37, DACO: M4.9

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1986094 Samuels, G.J. 1996. Trichoderma: a review of biology and systemics of the genus. Mycol. Res, 100: 923-935, DACO: M4.9

1807267 2009, Product Chemistry of BW240 WP, DACO: M2.10.3,M2.11,M2.12,M2.7.1, M2.7.2,M2.8,M2.9,M2.9.1,M2.9.2,M2.9.3

1951196 2010, Supplemental Product Chemistry of BW240 WP, DACO: M2.9.1 CBI

1951198 2010, Storage Stability, DACO: M2.11 CBI

2108995 2011, Response to Clarification Email, DACO: 0.8.1

2108997 Revised SOP 15-00 Fungal Contamination, DACO: M2.14 CBI

2108999 Revised SOP 14-00 Bacterial Contamination, DACO: M2.14 CBI

2122823 Part M2 email clarification.

2.0 Human and Animal Health

PMRA No. **Title**

1807268 2009, Acute Dermal Toxicity Study in Rats, DACO: M4.4

1807269 2009, Acute Dermal Irritation Study in Rabbits, DACO: M4.5.2

1807270 2009, Acute Oral Toxicity Study (UDP) in Rats, DACO: M4.9

1807271 2009, Acute Inhalation, DACO: M4.9

1807272 2009, Acute Eye Irritation in Rabbits, DACO: M4.9

1986035 2009, Acute Oral Toxicity, DACO: M4.9

1986037 2009, Acute Oral Toxicity CBI Reference Document, DACO: M4.9 CBI

1986038 2009, Response to Tier 1 Microbial Pesticide Data Requirements for G-41 Technical: Waiver for Acute Oral toxicity and pathogenicity TGAI, DACO: M4.2.2

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B. Additional Information Considered

i) Published Information

1.0 Methods of Analysis

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